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Short communication

Suppression of neuropathic pain by a naturally-derived peptide with NMDA antagonist activity

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Abstract

Chronic pain may result from hyperexcitability following activation of spinal NMDA receptors. A naturally-derived mammalian peptide, histogranin, may possess NMDA antagonist activity. This study explored the possibility that stable analog [Ser[1]]Histogranin (SHG) could reduce chronic pain. Neuropathic pain was induced using the chronic constriction injury model (CCI). Intrathecal injection of SHG markedly attenuated the hyperalgesia and allodynia resulting from CCI, nearly normalizing responses. These results suggest that the natural peptide histogranin may be a novel adjunct in neuropathic pain management.

Keywords: *N*-Methyl-D-aspartate (NMDA) receptor; Chronic pain; Spinal cord; Adrenal medulla; Peripheral neuropathy; Neuropeptide

Abnormal persistent pain is thought to involve a cascade of pathological events initiated by activation of *N*-methyl-D-aspartate (NMDA) receptors in the spinal cord [3,5,6,26,27]. In particular, neuropathic pain behaviors resulting from peripheral nerve injury are reduced by competitive and non-competitive NMDA antagonists [4,16,17,24,28]. This has led to preliminary clinical trials in neuropathic pain patients, a population that has been found to be less responsive to traditional pharmacotherapies. NMDA receptor blockade in patients suffering from neuropathic pain syndromes reduces ongoing pain and allodynia, although psychomimetic side effects are common [7,8,18,23].

Work in our laboratory has demonstrated that transplants of adrenal medullary chromaffin cells in the spinal subarachnoid space can reduce neuropathic pain symptoms, including allodynia and hyperalgesia, in animals with peripheral nerve injury [10,11]. In addition, these transplants appear to reduce pathological processes in the spinal cord of nerve-injured animals, including the loss of NMDA receptors, induction of NO synthase, and damage to dorsal horn inhibitory interneurons [9,12,14]. While chromaffin cells were initially selected for their secretion of potential pain-reducing agents, notably catecholamines and opioid peptides, recent findings have revealed that adrenal medullary extracts also contain a peptide with potential NMDA receptor antagonist activity [15]. The pentadecapeptide, histogranin, is associated with the chromaffin granule fraction, suggesting a neuropeptide function [15]. Histogranin has also been localized in the pituitary, brain, and blood plasma and both the natural peptide and its stable analog [Ser[1]]Histogranin (SHG) can block NMDA-induced convulsions in a dose-dependent fashion when injected i.c.v. [15,20]. In rat brain membrane preparations, SHG displaces the binding of ligands of the NMDA receptor [15] and [^{125}I]SHG binding is regionally distributed with higher levels in brain regions known to possess high NMDA receptor densities [19]. Together, these data suggest that histogranin may be an endogenous modulator of NMDA receptor functions. The goal of this study was to determine whether histogranin can attenuate neuropathic pain symptoms consequent to peripheral nerve injury. Preliminary findings from this study were presented previously [21].

All procedures involving animals were reviewed and approved by the institutional animal care committee. Male Sprague–Dawley rats (Sasco, WI) weighing approximately 250 g at the beginning of the study were used. Preligation responses to noxious and innocuous stimuli were determined as described in detail elsewhere [10,11,22]. Re-

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sponses to a noxious thermal stimulus were determined using a modification of the Hargreave's test [13]. Animals were placed beneath an inverted plastic cage on an elevated glass floor and a radiant heat source under the glass was aimed at the plantar hindpaw. Testing was alternated between hindpaws for three trials each with at least 30 s intertrial intervals and withdrawal latencies (determined electronically) were averaged for each hindpaw. A cutoff time of 15 s was used to avoid tissue damage in the absence of a response. Responses to noxious mechanical stimuli were assessed using the Randall-Selitto test. Pressure was applied to the plantar surface of each hindpaw sequentially at a constant rate of 64 g/s using a commercially available apparatus (Ugo-Basile), until the animal reacted with a withdrawal response. The apparatus automatically terminates at a scale reading of 25 (= 1000 g). Responses to innocuous tactile stimuli were assessed with a calibrated series of von Frey hairs ranging from 0.69 to 75.86 g using procedures similar to that described by others [25]. Animals were placed beneath an inverted clear plastic cage on an elevated mesh floor and von Frey hairs were indented on the hindpaw midplantar skin 5 times in rapid succession. Testing was alternated between both hindpaws using increasing von Frey hair forces until the animal responded with a paw withdrawal. The lowest hair in the series that evoked at least one withdrawal response was recorded as threshold.

Following baseline assessment, peripheral neuropathy was induced by unilateral chronic constriction nerve injury (CCI) described in detail previously [2,10,11]. Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p., supplemented as necessary), and the right common sciatic nerve was exposed at the mid-thigh level using blunt dissection. Four 4-0 chromic gut ligatures spaced about 1 mm apart were loosely tied around the sciatic nerve proximal to the trifurcation in order to constrict the nerve to a barely discernible extent. For intrathecal drug delivery, animals were also implanted with intrathecal catheters via a slit in the atlanto-occipital membrane and threaded through the spinal subarachnoid space to the level of the lumbar enlargement. The musculature was closed with silk sutures and the skin closed with wound clips. Animals were housed individually following surgical procedures, and allowed free access to food and water.

Behavioral responses were again assessed 2 weeks following CCI induction. Following determination of baseline responses to noxious and innocuous stimuli, animals received an intrathecal injection of SHG (1.0, 2.0, or 4.0 μg; $n = 8$ per dose). SHG was custom synthesized by Research and Diagnostic Antibodies (Berkeley, CA), dissolved in saline, and injected in 15 μl volumes followed by a 10 μl saline flush. A vehicle control was not included due to practical limitations in the number of animals feasibly tested within the time period, and since previous findings have indicated that saline does not alter pain responses. Behavioral responses were reassessed at 15, 30, 45, and 60 min following intrathecal injection. Statistical comparisons were made using ANOVA (repeated measures) and the Newman–Keuls test for multiple post-hoc comparisons.

Results are shown in Fig. 1 for responses to noxious thermal (Fig. 1A), noxious mechanical (Fig. 1B) and in-

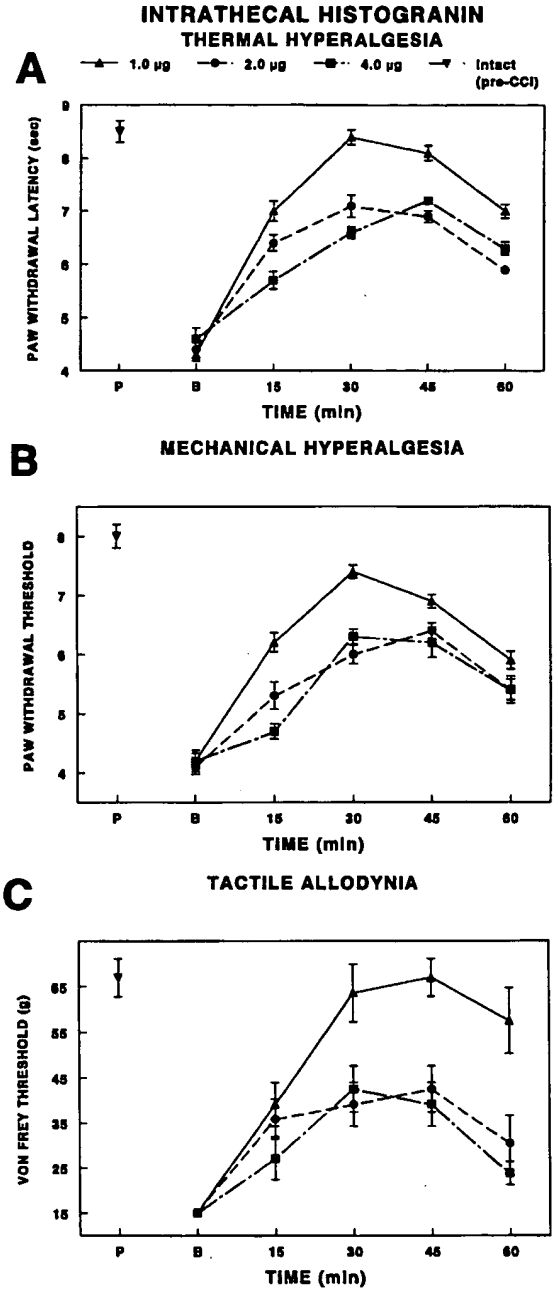

Fig. 1. Effects of intrathecally injected [Ser^1]Histogranin on responses to noxious thermal (A), noxious mechanical (B), and innocuous tactile (C) stimuli in animals with chronic constriction nerve injury (CCI). Responses in intact animals prior to nerve injury (at P; inverted triangles, pre-CCI) are shown for comparison. Mean ± S.E.M. responses are shown before histogranin (B) and at 15, 30, 45, and 60 min following intrathecal injection of histogranin at several doses (1.0 μg, ▲; 2.0 μg, •; 4.0 μg, ■; $n = 8$ animals per dose).

nocuous tactile (Fig. 1C) stimuli. Pre-ligation responses are shown for comparison ($n = 24$). Responses on the ligated side only are shown for clarity, since contralateral responses were not altered through the course of the study. Two weeks following CCI, baseline pain responses were determined (at 'B'), and animals were divided into three groups for intrathecal SHG injection ($n = 8$ per group). Prior to intrathecal histogranin, thermal and mechanical hyperalgesia and tactile allodynia were observed on the ligated side in animals with CCI, as described in other studies [1,2,10,11,14]. The intrathecal injection of SHG attenuated thermal hyperalgesia at all three doses tested (overall F (df 2,4) = 141.1, $F < 0.001$). Increased withdrawal latencies were apparent by 15 min following injection ($P < 0.05$ compared to baseline for all three doses). Peak reductions in hyperalgesia occurred approximately 30 min following intrathecal injection, with a tendency towards pre-injection latencies at later times. Interestingly, the lowest dose of SHG utilized appeared to produce the most potent anti-hyperalgesic effect ($P < 0.05$ compared to both higher doses). At its peak, this dose of intrathecal SHG nearly completely restored paw withdrawal latencies to those of intact, non-ligated animals.

Intrathecal SHG similarly reduced mechanical hyperalgesia in CCI animals (Fig. 1B; overall F (df 2,4) = 124.04, $F < 0.001$). Again, although all three doses of SHG produced significant anti-hyperalgesic effects ($P < 0.05$ compared to baseline), the 1.0 μg dose was the most potent ($P < 0.05$ compared to the other doses). The peak effects occurred at 30 min post-injection, with a tendency toward baseline at later time points. Tactile allodynia was also attenuated by intrathecal SHG (Fig. 1C; overall F (df 2,4) = 23.76; $P < 0.001$; $P < 0.05$ for all three doses compared to baseline). The 1.0 μg dose was the most potent ($P < 0.05$ compared to the other doses), reversing the allodynia nearly to pre-ligation tactile response thresholds.

Results of this study demonstrate that SHG, a stable analog of naturally-derived peptide histogranin, can reverse hypersensitivity to noxious and innocuous stimuli brought about by peripheral nerve injury. In particular, the optimal dose identified, 1.0 μg nearly completely eliminated thermal and mechanical hyperalgesia and tactile allodynia at the time of its peak activity. Higher doses also attenuated hyperalgesia and allodynia, but to a lesser degree, suggesting the possibility of partial agonist activity or recruitment at feedback or compensatory sites. Histogranin is particularly interesting as a potential therapeutic agent as it has been localized in the pituitary, brain, and adrenal medulla, suggestive of a neuropeptide profile that may be an endogenous modulator of NMDA receptor functions [15,20]. In animal models, histogranin and SHG can block NMDA-induced convulsions and binding is localized to brain regions known to possess high NMDA receptor densities [19].

Recent theories regarding the induction and maintenance of abnormal persistent pain syndromes, in distinction from acute nociceptive transmission, suggest the involvement of activation of spinal NMDA receptors [3,5,6,26,27]. This is supported by findings in animal models demonstrating a reduction in hyperalgesia by NMDA antagonists such as ketamine, MK-801, and dextrorphan [4,16,17,25,28], and has led to preliminary clinical trials using NMDA antagonists [7,8,18,23]. As neuropathic pain patients tend to be less responsive to traditional pain pharmacotherapies, these results have been promising. However, psychomimetic side effects are commonly reported with systemic administration of these agents. Since histogranin is a peptide, it is unlikely that systemic administration will be effective, and findings from other laboratories have demonstrated that i.c.v. administration produces dose-dependent, phencyclidine-like stereotypy, ataxia, and locomotor activity, suggesting a profile consistent with other NMDA antagonists [20]. In contrast, similar side effects were not observed in the present study when SHG was administered intrathecally. Thus, it is likely that there is negligible activity at higher centers following intrathecal administration due to limited diffusion and rapid degradation of the active peptide.

In summary, results of this study demonstrate that a natural peptide, derived from neural tissue, can attenuate hyperalgesia and allodynia resulting from peripheral nerve injury when injected intrathecally, and that this may be a promising approach in the management of neuropathic pain with reduced toxicity.

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